Molluscicidal Activity and Biochemical Effects of Two Phyto-Glycosides against Land Snails

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ABSTRACT

The cardiac glycosides ouabain and lanatoside C were tested for their molluscicidal activity against two terrestrial snails - *Eobania vermiculata* (Müller) and *Theba pisana* (Müller). Lanatoside C did not exhibit any molluscicidal action against the two tested snails at the range of the tested doses (up to 80 µg/g body weight). Although, ouabain was inactive against *E. vermiculata*, it was highly toxic to *T.pisana*. The median lethal dose (LD_{50}) was 9.35 µg/g body weight after 24 hr of treatment. Sub-lethal doses of ouabain caused marked decrease in *T.pisana* protein concentration. Aspartate transaminase (AST) and alanin transaminase (ALT) activities were significantly stimulated by the tested doses of ouabain, especially the lower ones, indicating the lesion occurred in the snail's digestive gland. Response of ALT toward ouabain treatment was more sensitive than AST.

INTRODUCTON

Terrestrial gastropod mollusk species are currently considered of the most significant threats to sustainable agriculture in many parts of the world (Barker, 2002). Synthetic molluscicides are the main mollusks control method, but environmental hazards of these chemicals (Buchs *et al.*, 1989) directed researchers to find out environmentally safer alternative control tools. Although large numbers of phytochemicals have already been isolated and shown to have molluscicidal activity against aquatic snails (Mott, 1987; Thiilborg *et al.*, 1993; Lemmich *et al.*, 1995; Ekabo *et al.*, 1996). A little work has been carried out to evaluate natural molluscicides against terrestrial mollusks and few numbers of natural compounds of plant origin were proved to be biologically active against terrestrial gastropod species (Hussein and El-Wakil, 1996; El-Zemity and Radwan, 2001; Hussein, 2005; El-Zemity, 2006). Ouabain (g-strophanthin) is standard cardiac glycoside found in the ripe seeds of African plants *Strophanthus gratus* and *Acokanthera ouabaio*. Lanatoside C is another cardiac glycoside found in *Digitalis lanata* (Scheiner-Bobis and Schoner, 2001). Very few glycoside extracts or compounds were proved to be active molluscicides against land snails (Hussein *et al.*, 1994 and 1999). Continuing to our effort to find out natural molluscicides we have choised the standard cardiac glycosides, ouabain and lanatoside C to be tested for

their molluscicidal activity. This is the first report on the molluscicidal activity of these compounds.

Key words: Ouabain, lanatoside C, cardiac glycosides, molluscicidal activity, land snails.

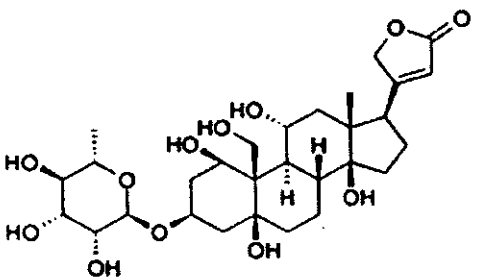
MATERIALS AND METHODS

1- Snails

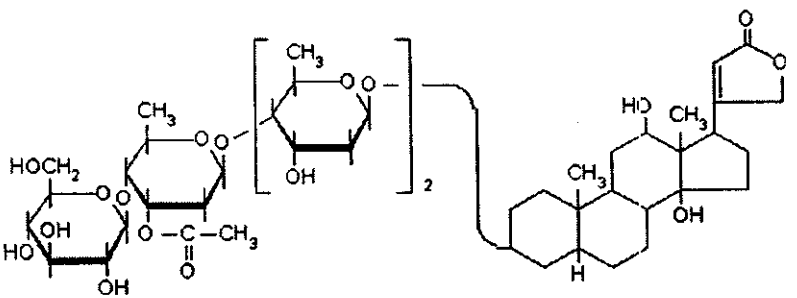
Adult terrestrial helicid snails *Theba pisana* (Müller) and *Eobania vermiculata* (Müller) were collected from pesticide-free garden in Noubaria. Snails were reared for an enough long period to be fully acclimatized to laboratory conditions prior to the test.

2- Chemicals

Tow cardiac glycoside compounds, ouabain (g-strophanthin) 95% (Fig.1-a) and lanatoside C 97% (Fig. 1-b) were purchased from Sigma.



(a)



(b)

Fig (1): Chemical structure of tested cardiac glycosides (a) ouabain and (b) lanatoside C.

3- Bioassay

Stock solutions of both tested compounds were prepared by dissolving each compound in dimethylsulfoxide (DMSO), which was diluted with water. Tween 80 was added to prevent precipitation. Concentrated stocks were diluted with water to obtain the lower doses. Snails as a check control were treated with the solvent. Three replicates were used for each dose with 10 snails each. Tested dose was gently applied on the surface of the snail mantle collar using a micropipette as the method described by Hussein *et al.*, (1994). Snails were fed on lettuce *ad libitum*. Dead snails were detected 24 hr after treatment by loss of response to a thin stainless steel needle (WHO, 1965).

4- Sub-lethal treatments

Snails were treated with1/2, 1/5 and 1/10 of LD_{50} value of molluscicidal active compound(s). After 24h of treatment, snails' tissues were prepared for biochemical tests.

5- Sample preparation for biochemical tests

Shells of snails were removed and the isolated body tissues were homogenized in 10 folds (w/v) of distilled water by using glass homogenizer. Homogenates were centrifuged at 5000 rpm for30minutes using a cooling centrifuge at 4 °C. The supernatant was used as a source of protein and enzyme assay.

6- Determination of protein content

Estimation of protein concentration has been carried out according to the method of Lowery *et al.* (1951) using bovine serum albumin as standard and absorbance was measured at 750 nm.

7- Aspartate transaminase (AST) and alanin transaminase (ALT) assay

In vivo effects of molluscicidal active compound(s) against AST and ALT were assessed according to the method described by Reitman and Frankel (1957) using Diamond Diagnostics kit. Absorbance was measured at 546 nm.

8- Statistical analysis

Toxicity values were determined by probit analysis (Finney, 1971). Analysis of variance (ANOVA) was used with enzyme assay and significant differences between means were calculated by least significant difference (LSD) at $p = 0.05$.

RESULTS AND DISCUSSION

1- Molluscicidal activity

Results, concerning the molluscicidal activity of tested cardiac glycosides, revealed that lanatoside C was entirely ineffective against both of tested snails : *E. vermiculata* ond *T. pisana* in the range of the tested doses (no mortalities up to 80 µg/g body weight). Ouabain also was inactive against *E. vermiculata* (LD_{50}> 80 µg/g body weight), but it was highly toxic to *T.pisana* by contact as shown in Table (1). Toxicity values LD_{50} and LD_{90} after 24h of ouabain treatment were 9.35 and 19.07 µg/g body weight respectively. Our findings indicate that ouabain has high molluscicidal activity against *T. pisana* compared to the carbamate compound, methomyl which has LD_{50} value 114.23 µg/g body weight as reported by Hussein and El-Wakil (1996). Although *E. vermiculata* and *T. pisana* belong to the same family, Helicidae and subfamily, Helicinae (Godan, 1983), ouabain exhibited species selectivity as it is very toxic against only one of them.

There is numerous literary information on pesticidal effects of plant extracts containing cardiac glycosides or their constituents against different pests like, insects (Morsy *et al.*, 2001), nematodes (Latif *et al.*, 1999), bacteria (Awadh, 2001), fungi (Yasmeen, 2000), ticks (Al-Rajhy *et al.*, 2003), mollusks (Hussein *et al.* 1994) and rodents (Meister, 1992). However there is no data on molluscicidal properties of the cardiac glycoside ouabain. This is the first prove of ouabain molluscicidal activity against terrestrial gastropod mollusks. The molluscicidal action of ouabain was rapid, symptoms of toxicity appeared after 1-2 h of treatment. Snails retracted their bodies inside the shells; the ends of their feet protrude and lie on the surface of shell aperture and died in this position within 24 hr of treatment. These symptoms are similar to those described by Hussein *et al.* (1999) who used the cardiac glycoside extract from the plant *Pergularia tomentosa* (L.) against the land snail *Monacha obstructa.*

2- Biochemical effects

It is important to study the influence of toxic compounds on the biochemical systems in the target pest. Sub-lethal doses of 1/10, 1/5 and 1/2 ouabain LD_{50} reduced protein level as presented in Table (2). Marked decrease of protein levels was observed due to treatment with lower doses of 0.93 and 1.87 µg/g body weight which caused 44 and 43 reduction percentages, respectively, compared to control. Many authors reported on the reduction of mollusks' protein level as a result of toxicosis by different toxicants either synthetic or natural (Adewunmi *et al.*, 1988; Mohammed *et al.*, 1981; Chaudhary and Lomte, 1990; El-Wkil and Radwan, 1991).

Transaminases (aminotransferases) constitute a group of enzymes that catalyze the interconversion of amino acid in α- keto acid by transferring amino group (Moss and Henderson, 1998). Aspartate transaminase (AST) and alanin transaminase (ALT) are enzymes located in molluscan digestive gland (hepatopancreas) that leak out into general circulation when hepatic cells injured. The transaminases are good indicators of tissue lesion. Results of *in vivo* effect of ouabain on *T. pisana* AST and ALT activities are presented in Table (3) and illustrated in Figures (2 and 3).Significant increases were observed in both enzymes especially with lower doses of 1/10 and 1/5 LD_{50} value (Table 3). Elevation of ALT activity reached 2 and 3 folds (Figure 3) compared to control. Maximum increase in AST activity was 1.6 fold (Figure 2) with 1/10 of ouabain LD_{50} value. ALT was more sensitive than AST to ouabain treatments. Elevation of AST and ALT activities in treated terrestrial snails with compounds possessing molluscicidal properties has been reported in the works of El-Wakil and Radwan (1991) and Abdallah *et al.* (1998).

The mechanism of ouabain rapid molluscicidal action is unknown so, further studies on its mode of action is needed. The relation between cell death and ouabain classical action in Na^+, K^+-ATPase inhibition became uncertain according to the report of Valente *et al.* (2003). Their results have three major implications: first, there is no direct correlation between membrane depolarization and ouabain-induced cell death; second, ouabain toxicity is related to signaling transduction and is not directly related to its classical action in Na^+, K^+-ATPase inhibition and third, GSH play a major role in ouabain-induced signaling transduction and cell death. Also, the question that needs an answer is why ouabain was very toxic against only *T. pisana* and did not exhibit any toxicity against *E. vermiculata*? So, a lot of biochemical and molecular based work is needed to answer these questions.

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Table (1): Toxicity of ouabain against *Theba pisana*.

Mortality (%)	Lethal Dose (µg/g b.w)		
	Mean	Lower Limit	Upper Limit
25	6.42	5.59	7.16
50	9.35	8.48	10.28
75	13.60	12.24	15.50
90	19.07	16.59	23.05

Slope: 4.14 ± 0.396 *$(Chi)^2$: 0.03*

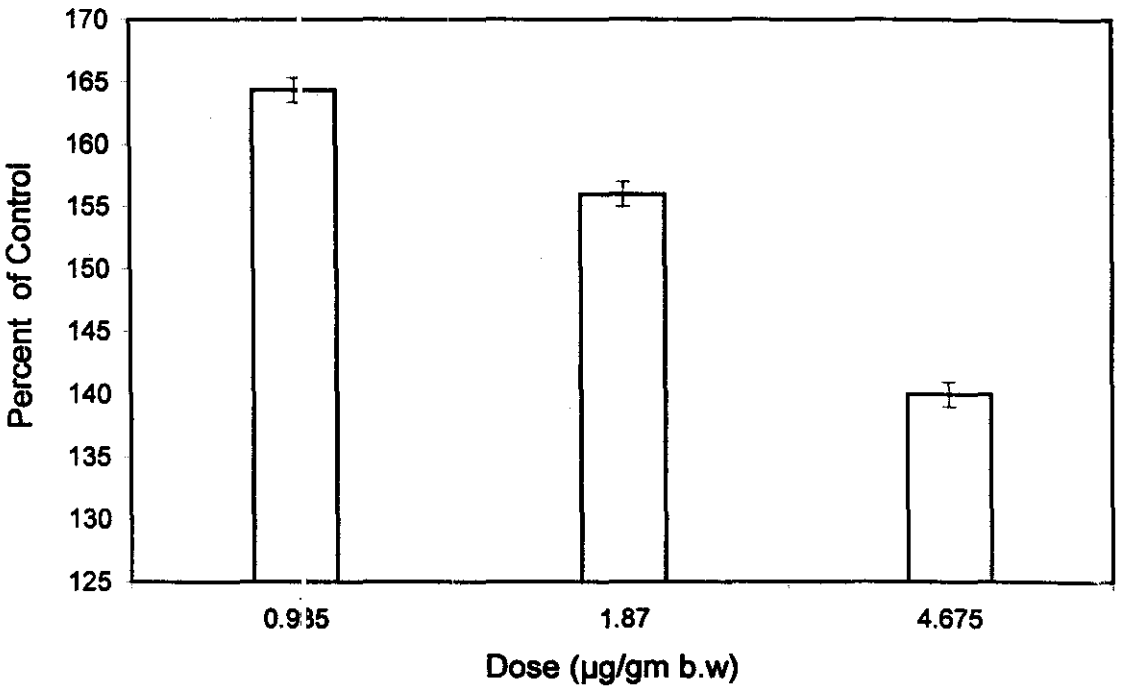
Table (2): Effect of ouabain on protein content of *Theba pisana* (mg protein ml^{-1}).

Dose (µg/g b.w)	Mean ± SD	% of Control
Control	9.30 ± 0.0	-
0.935	5.21 ± 0.087	56
1.870	5.30 ± 0.175	57
4.675	6.11 ± 0.184	66

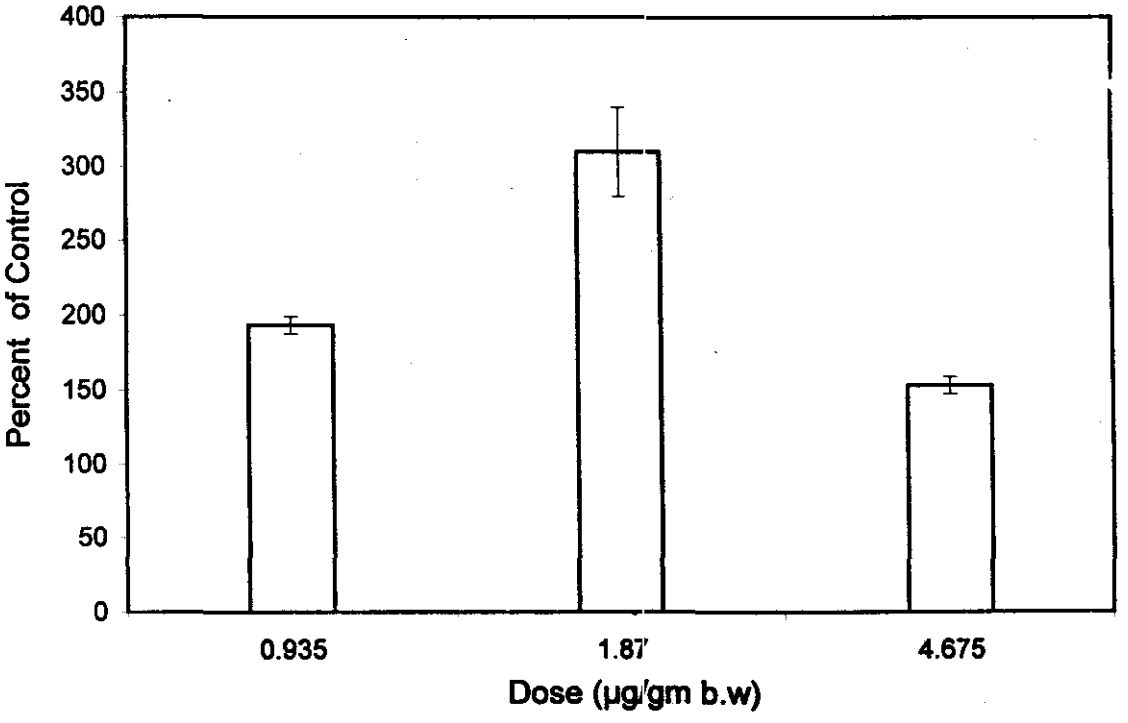
Table (3): The assed activity values of aspartate transaminase (AST) and alanin trnsaminase (ALT) after treatment of *Theba pisana* snail with Ouabain.

Dose (µg/g b.w)	Specific activity (OD mg protein^{-1} ± SD)	
	AST	ALT
Control	0.032 ± 0.0^{a}	0.01 ± 0.0^{a}
0.935	0.053 ± 0.0009^{b}	0.02 ± 0.0005^{c}
1.870	0.050 ± 0.0008^{b}	0.031 ± 0.002^{d}
4.675	0.045 ±0.0008^{ab}	0.015 ± 0.005^{b}
$L.S.D_{0.05}$	0.018	0.0046

Values with the same superscript(s) within the same column are not significant.



Fig(2): Effect of ouabain on *Theba pisana* AST activity.



Fig(3): Effect of ouabain on *Theba pisana* ALT activity.

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الملخص العربي

النشاط الإبادي والتأثيرات البيوكيميائية لإثنين من الجليكوسيدات النباتية ضد القواقع الأرضية.

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تم إختبار النشاط الإبادي لمركبين من الجليكوسيدات المستخدمة في علاج بعض أمراض القلب وهما الأوابين ولاناتوزايد(س) ضد نوعين القواقع الأرضية وهما القوقع البني إيوبانيا فيرميكيولاتا والقوقع الأبيض ثيبا بيسانا. أظهرت النتائج أن مركب لاناتوزايد(س) لم يظهر أي فاعلية ضد نوعي القواقع تحت الإختبارفي مدى الجرعات المستخدمة (حتى ٨٠ ميكروجرام/جرام من وزن الجسم). أما مركب الأوابين الذي لم يظهر أيضا فاعلية ضد القوقع البني كان شديد الفاعلية ضد القوقع الأبيض وكانت الجرعة القتلة لنصف القواقع المختبرة هي ٩,٣٥ ميكروجرام/جرام من وزن الجسم بعد ٢٤ ساعة من المعاملة. و أظهر إختبار الجرعات المنخفضة لمركب الأوابين انخفاض واضح لمستوى البروتين في القوقع الأبيض كما أحدثت هذه الجرعات زيادة معنوية في نشاط إنزيمي أسبارتيت ترانس أمينيز و ألانين ترانس أمينيزمقارنة بالمجموعة الضابطة مما يدل على حدوث خلل في الغدة الهاضمة.